TOXICITY TESTS ON BIOLOGICAL SPECIES INDIGENOUS TO THE GULF OF MEXICO

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ABSTRACT: *Dispersant toxicity tests were performed on marine species indigenous to the Gulf of Mexico in support of the Region VI Regional Response Team Dispersant Work Group. Concurrent with the toxicity testing, the group is developing a Subpart H regional contingency plan for authorization of the use of dispersants in non-life-threatening situations.*

Static and flowthrough toxicity tests were performed on five Gulf species: brown shrimp, white shrimp, blue crab, eastern oyster, and redfish.

The test results indicated that when the test species were exposed to chemically dispersed Mayan or Saudi Arabian Light (SABL) crude oil, the observed toxicity occurred rapidly, usually within 6 hours after addition of the dispersed oil. During the tests, the organisms became disoriented and lethargic immediately after addition of the dispersed crude oils. However, the test animals that survived the first 6 to 12 hours of exposure usually recovered and survived for the remainder of the test period. In almost all cases, surviving test organisms were swimming normally and were not lethargic 12 to 24 hours after test material addition.

Test results indicated that if the organisms were exposed to dispersed Mayan or SABL crude oil, but survived long enough for the material to decrease in concentration, the organisms could recover and survive without any apparent short-term effects.

Since 1986, the Region VI Regional Response Team (RRT) Dispersant Work Group (DWG) has been developing a Subpart H regional contingency plan for authorization of the use of dispersants in non-life-threatening situations. This document is consistent with the National Contingency Plan and is intended to be used in responding to oil spills within the Gulf of Mexico and contiguous coastal waters within Region VI (Texas and Louisiana).

Recognizing early that dispersant toxicity data were lacking on marine species in the Gulf of Mexico, LOOP, Inc., agreed to fund an effort to provide some of this data. Discussions with the DWG concluded that the following species would be tested: brown shrimp, white shrimp, blue crab, eastern oyster, and redfish. Two types of toxicity tests would be performed: static and flowthrough tests. The DWG further felt it was necessary that test results achieve a 96-hour LC_{50} value (lethal concentration for 50 percent mortality of individuals) for each species tested. Hence, concentrations of dispersed oil used in both static and flowthrough tests are higher than would be experienced in real world dispersant application.

Tests commenced in November of 1986 and were completed in August of 1988. The results that follow will be incorporated in Appendix G of the Subpart H contingency plan.

Materials and methods

Test materials. Two crude oils were used in testing. Samples of Mayan crude oil and Saudi Arabian Light (SABL) crude oil were provided by LOOP, Inc. The dispersant used in all tests was Exxon Corexit 9527.

Test water. The acclimation, dilution, and control water used in the tests was natural seawater collected from the Gulf of Mexico. The seawater was adjusted to 25 parts per thousand using deionized water and vigorously aerated prior to use in the toxicity tests.

Test animals. The test animals selected for the program were those that are indigenous to the Gulf of Mexico and adjacent estuaries and have economic importance in Gulf of Mexico fisheries. Test species were the blue crab (*Callinectes sapidus*), brown shrimp (*Penaeus aztecus*), white shrimp (*Penaeus setiferus*), eastern oyster (*Crassostrea virginica*), and redfish (*Sciaenops ocellatus*).

Test conditions. The tests were conducted in an environmental chamber in which temperature was maintained at 22 ± 2° C. Lighting was provided by cool white fluorescent bulbs on a 12-hour-light/12-hour-dark cycle. Test containers for all tests were 38 liter (L) glass aquaria which contained 28.5 L of test solution or control seawater. Liquid depth in the test chambers was 30 centimeters (cm)) (1 ft) and the surface area of each aquarium was 900 cm^2 (1 ft^2). Volume in each aquarium was 28,400 cm^3 (1 ft^3).

Seawater was added to each aquarium and aeration was provided through 1 mL TD-EX disposable glass pipettes and aquarium pumps. The test animals were then added to the aquaria. A minimum of ten animals were tested per aquarium and all treatments were duplicated, resulting in at least 20 animals per treatment. All animals were fed once daily during the test.

In flowthrough tests, a continuous flow of seawater was supplied to all aquaria throughout the test period. A 1,200 L holding tank inside the laboratory provided water to a constant pressure headbox with a 130 L capacity. Seawater level was held constant in the headbox by means of a float switch controlling an electric feed pump. From the headbox, seawater flowed through Teflon stopcocks. Flow rate to each aquarium was adjusted to approximately 60 mL per minute. The flow rate produced approximately a 50 percent volume change in each aquarium every 8 hours.

For the dispersed crude oil tests, a solution was prepared using 10 parts crude oil to 1 part dispersant (100 mL oil : 10 mL Corexit 9527). The oil and dispersant mixture was thoroughly mixed and test solutions were prepared by the addition of the appropriate volume of the stock solution directly to the aquaria. The chemical mixture was injected using airtight syringes directly into the aeration stream below the surface of the seawater to initiate the tests.

For the SABL crude oil test, the oil was also injected directly into the aeration stream below the surface of the seawater to initiate the tests.

During each dispersed crude oil test, a dispersant treatment was conducted concurrently and was usually the highest dispersant concentration used with crude oil in the test.

Statistical analyses. Based on the results of the tests, the LC_{50} values (the initial exposure concentrations of crude oil that produced 50 percent mortality after a specific period of exposure) and their associated 95 percent confidence limits were calculated. The computer program estimated LC_{50} values using the following statistical methods: moving average angle, probit analysis, and binomial probability.[2] The method selected for reporting the results of the test was determined by the characteristics of the data, that is, the presence or absence of 0 and 100 percent mortality and the number of concentrations in which mortality between 0 and 100 percent occurred.[1]

The oyster shell deposition test data were analyzed using analysis of variance (ANOVA) and Dunnett's test. ANOVA indicated whether there was a statistically significant difference between the control and any of the crude oil treatments. Dunnett's test indicated which crude oil concentrations were statistically different, at the 95 percent confidence level, from the control.

Results

Toxicity tests with dispersed Mayan crude oil

Blue crab. A static, 96-hour toxicity test was conducted from November 13 through 17, 1986, using the blue crab (*Callinectes sapidus*). Ten crabs were used per duplicate resulting in 20 crabs exposed per test treatment. Test concentrations were 15, 30, 75, 150, 210, and 300 parts per million (ppm) dispersed crude oil. The carapace width of the test crabs ranged from 1.4 to 2.6 cm; mean carapace width was 2.0 ± 0.3 cm (Table 1).

After 24 hours of exposure 100 percent mortality had occurred in 300 ppm dispersed Mayan crude oil. The 24-hour LC_{50} was 54 ppm with 95 percent confidence limits of 30 to 75 ppm. After 96 hours of exposure, mortality was 0, 10, 85, 100, 95, and 100 percent in 15, 30, 75, 150, 210, and 300 ppm dispersed Mayan crude respectively. Five percent mortality occurred in the seawater control and 30 ppm Corexit 9527 treatment. The 96-hour LC_{50} was 49 ppm with 95 percent confidence limits of 30 to 75 ppm. After 96 hours of exposure, no statistically significant mortality occurred in test concentrations less than and equal to 30 ppm dispersed Mayan crude oil (Tables 2 and 3).

Table 1. Test species, test dates, and lengths of animals used in toxicity tests with chemically dispersed Mayan crude oil

Test species	Test dates	Animal length (cm) Mean ± SD_1	Range
Callinectes sapidus	13–17 Nov 86	2.0 ± 0.3	1.4–2.6
Callinectes sapidus	23–27 Apr 86	2.1 ± 0.3	1.6–2.6
Penaeus aztecus	20–24 May 87	3.2 ± 0.6	2.5–4.8
Penaeus setiferus	21–25 Aug 87	4.6 ± 0.9	2.4–5.9
Crassostrea virginica	02–09 Dec 87	3.3 ± 0.4	2.6–4.1
Sciaenops ocellatus	06–10 Jan 88	3.4 ± 0.3	2.9–3.7

1. SD = standard deviation

A flowthrough, 96-hour toxicity test was conducted from April 23 through 27, 1987, using the blue crab. Fifteen crabs were used per duplicate, resulting in 30 crabs per test treatment. Test concentrations were 25, 50, 75, 100, and 150 ppm dispersed crude oil. The carapace width of the test crabs ranged from 1.6 to 2.6 cm; mean width was 2.1 ± 0.3 cm (Table 1).

After 96 hours of exposure, maximum mortality was 47 percent in 150 ppm dispersed Mayan crude oil. The 96-hour LC_{50} was greater than 150 ppm. No statistically significant mortality occurred in test concentrations less than or equal to 50 ppm dispersed Mayan crude (Tables 4 and 3).

Brown shrimp. A flowthrough 96-hour toxicity test was conducted from May 20 through 27, 1987, using the brown shrimp (*Penaeus aztecus*). Ten shrimp were tested per duplicate, resulting in 20 shrimp per test treatment. Test concentrations were 9.4, 18.8, 37.5, 75, and 150 ppm dispersed oil. Length of the test shrimp ranged from 2.5 to 4.8 cm; mean length was 3.2 ± 0.6 cm (Table 1).

After 6 hours of exposure, 100 percent mortality had occurred in 75 and 150 ppm dispersed crude oil. The 6-hour LC_{50} was 36 ppm, with 95 percent confidence limits of 18.8 to 75 ppm dispersed Mayan crude oil. No statistically significant mortality occurred in test concentrations less than or equal to 18.8 ppm (Tables 5 and 3).

Table 2. Mortality of blue crab exposed to chemically dispersed Mayan crude oil in a static acute test

Oil : dispersant test concentration (ppm)	Rep	Cumulative mortality (percent) 24 h	48 h	72 h	96 h
Seawater control	A	0	0	10	10
	B	0	0	0	0
0 : 30	A	0	0	0	0
	B	10	10	10	10
15 : 1.5	A	0	0	0	0
	B	0	0	0	0
30 : 3.0	A	0	0	10	10
	B	0	0	10	10
75 : 7.5	A	90	90	90	90
	B	80	80	80	80
150 : 15	A	100	—	—	—
	B	70	90	90	100
210 : 21	A	100	—	—	—
	B	70	90	90	90
300 : 30	A	100	—	—	—
	B	100	—	—	—

Table 3. Summary of results of toxicity tests using chemically dispersed Mayan crude oil

Test species	Type test[1,2]	LC_{50} value (ppm) or effect measured[3]					
		1 h	6 h	24 h	48 h	72 h	96 h
Callinectes sapidus	S	NEM	NEM	54	54	49	43
Callinectes sapidus	FT	>150	>150	>150	>150	>150	>150
Penaeus aztecus	FT	NEM	47	41	36	36	36
Penaeus setiferus	FT	NEM	50	45	45	44	44
Penaeus setiferus (30 days)	SR	No significant difference in mortality, length, or dry weight					
Crassostrea virginica (7 days)	FT	One mortality and significant reduction of shell growth at 2,500 ppm					
Sciaenops ocellatus	FT	NEM	418	387	387	387	387

1. S = static
2. FT = flowthrough
3. NEM = not enough mortality to calculate an LC_{50} value

Table 4. Mortality of blue crab exposed to chemically dispersed Mayan crude oil in a flowthrough acute test

Oil : dispersant test concentration (ppm)	Rep	Cumulative mortality (percent)				
		6 h	24 h	48 h	72 h	96 h
Seawater control	A	0	0	0	0	0
	B	0	0	0	0	0
0 : 15	A	0	0	0	0	0
	B	0	0	0	0	0
25 : 2.5	A	0	0	0	0	0
	B	0	0	0	0	0
50 : 5	A	0	0	6.6	6.6	6.6
	B	0	6.6	13.3	13.3	13.3
75 : 7.5	A	6.6	6.6	13.3	13.3	13.3
	B	0	0	6.6	13.3	13.3
100 : 10	A	0	0	0	6.6	13.3
	B	0	0	20.0	20.0	20.0
150 : 15	A	6.6	33.0	53.0	53.0	53.0
	B	13.3	26.6	33.3	40.0	40.0

Table 5. Mortality of brown shrimp exposed to chemical dispersed Mayan crude oil in a flowthrough acute test

Oil : dispersant test concentration (ppm)	Rep	Cumulative mortality (percent)				
		6 h	24 h	48 h	72 h	96 h
Seawater control	A	0	0	0	0	0
	B	0	0	0	0	0
0 : 150	A	0	30	40	40	40
	B	0	20	40	40	40
9.4 : 0.94	A	0	0	0	0	0
	B	0	0	0	0	0
18.8 : 1.88	A	0	0	0	0	0
	B	0	0	0	0	0
37.5 : 3.75	A	20	40	60	60	60
	B	20	40	50	50	50
75 : 7.5	A	100	—	—	—	—
	B	100	—	—	—	—
150 : 15	A	100	—	—	—	—
	B	100	—	—	—	—

White shrimp. A flowthrough, 96-hour toxicity test was conducted from August 21 through 25, 1987, using the white shrimp (*Penaeus setiferus*). Twelve shrimp were tested per duplicate, resulting in 24 shrimp per test treatment. Test concentrations were 9.4, 18.8, 37.5, 75, and 150 ppm dispersed Mayan crude oil. Length of the test shrimp ranged from 2.4 to 5.9 cm; mean length was 4.6 ± 0.90 (Table 1).

After 6 hours of exposure, 100 percent mortality had occurred in 75 and 150 ppm conditions. The 6-hour LC_{50} was 50 ppm, with 95 percent confidence limits of 18.8 to 75 ppm. The 96-hour LC_{50} was 44 ppm, with 95 percent confidence limits of 18.8 to 75 ppm. No statistically significant mortality occurred in test concentrations less than or equal to 18.8 ppm dispersed Mayan crude oil (Tables 6 and 3).

A follow-up study was conducted on the white shrimp exposed to dispersed Mayan crude oil. The 30 day post-exposure study was conducted from August 26 through September 24, 1987, and was a static, daily renewal procedure with approximately 50 percent of the seawater replaced daily. Test animals previously exposed to the seawater control, 9.4, 18.8 and 37.5 ppm dispersed Mayan crude oil and 15 ppm Corexit 9527 were observed during the 30 day period. The initial number of organisms on day 0 of the recovery study were 24 in the seawater control; 23, 24, and 18 in the 9.4, 18.8, and 37.5 ppm dispersed Mayan crude conditions respectively, and 24 in the 15 ppm Corexit 9527 condition. On day 30, the number of live shrimp in the same treatments were 24, 22, 23, 18, and 22. Comparing survival, length, and dry weight of control and exposed shrimp after the 30 day post-exposure period demonstrated no statistically significant difference between control animals and those previously exposed to dispersed Mayan crude oil (Table 7).

The lengths of shrimp that died in the 75 and 150 ppm Mayan crude oil treatments within 6 hours of exposure were not statistically different from the lengths of shrimp after the 30 day post-exposure study. However, dry weights of the animals doubled or tripled after the 30 day exposure.

Eastern oyster. A flowthrough, seven day test was conducted from December 2 through 9, 1987, using the eastern oyster (*Crassostrea virginica*). Twenty oysters were used per duplicate, resulting in 40 oysters per test treatment. Test concentrations were 156, 312, 625, 1,250, and 2,500 ppm dispersed Mayan crude oil. Length of the test oysters at initiation of the test ranged from 2.6 to 4.1 cm; mean length was 3.3 ± 0.4 cm (Table 1).

After seven days of exposure, one mortality occurred in 2,500 ppm dispersed oil. No mortality occurred in any other test concentration or the seawater control. A statistically significant reduction in shell deposition occurred among oysters exposed to 2,500 ppm dispersed crude oil compared to the seawater control (Tables 8 and 3).

Redfish. A flowthrough, 96-hour toxicity test was conducted from January 6 through 10, 1988, using the redfish (*Sciaenops ocellatus*). Ten fish were tested per duplicate, resulting in 20 fish per test treat-

Table 6. Mortality of white shrimp exposed to chemically dispersed Mayan crude oil in a flowthrough acute test

Oil : dispersant test concentration (ppm)	Rep	Cumulative mortality (percent)				
		6 h	24 h	48 h	72 h	96 h
Seawater control	A	0	0	0	0	0
	B	0	0	0	0	0
0 : 15.0	A	0	0	0	0	0
	B	0	0	0	0	0
9.4 : 0.94	A	0	0	0	0	0
	B	8.3	8.3	8.3	8.3	8.3
18.8 : 1.88	A	0	0	0	0	0
	B	0	0	0	0	0
37.5 : 3.75	A	0	16.6	16.6	16.6	16.6
	B	16.6	33.3	33.3	41.6	41.6
75 : 7.5	A	100	—	—	—	—
	B	100	—	—	—	—
150 : 15	A	100	—	—	—	—
	B	100	—	—	—	—

Table 7. Survival, length, and dry weight of white shrimp after 30 days post-exposure study

Oil : dispersant initial exposure concentration (ppm)	Rep	Number of live shrimp		Animal length (cm)[1,2]		Animal dry weight (mg)[1,2]	
		Initial	Final	X (± SD)	Range	X (± SD)	Range
Seawater control	A	12	12	4.7 (1.0)	3.0–5.6	124 (58)	40–202
	B	12	12	4.5 (0.5)	3.5–5.2	105 (41)	46–168
0 : 15	A	12	10	4.9 (0.7)	3.1–5.5	158 (52)	41–218
	B	12	12	4.8 (0.7)	3.3–5.7	161 (56)	48–222
9.4 : 0.4	A	12	12	4.8 (0.8)	3.0–6.2	138 (69)	40–307
	B	11	10	5.2 (0.4)	4.5–5.9	159 (35)	100–227
18.8 : 1.88	A	12	11	4.6 (0.6)	3.6–5.6	117 (48)	41–203
	B	12	12	4.5 (0.8)	2.9–6.0	128 (69)	30–296
37.5 : 3.75	A	11	11	4.9 (0.4)	4.1–5.8	136 (48)	70–254
	B	7	7	5.3 (0.5)	4.2–5.8	190 (44)	96–224

1. X = mean
2. SD = standard deviation

Table 8. Eastern oyster shell deposition after seven days in flowthrough acute toxicity test of dispersed Mayan crude oil

Oil : dispersion test concentration (ppm)	Rep	Shell deposition (mm)[1,2] X	SD	Range
Seawater control	A	6.5	4.1	3.1–10.1
	B	5.9	3.1	2.9–9.0
0 : 250	A	5.9	1.5	3.3–7.9
	B	4.9	2.6	1.9–7.9
156 : 15.6	A	5.7	3.5	0.0–9.1
	B	5.6	2.3	4.0–8.4
312 : 31.2	A	6.1	2.0	3.4–8.6
	B	6.0	2.7	2.9–8.9
625 : 62.5	A	5.1	3.0	2.0–8.1
	B	5.6	4.4	0.0–8.9
1250 : 125	A	5.8	5.5	1.1–10.1
	B	4.3	3.3	1.1–7.8
2500 : 250	A	4.2	5.1	0.0–8.4
	B	3.0	5.3	0.0–6.1

1. X = mean value
2. SD = standard deviation

ment. Test concentrations were 65, 110, 180, 300, and 500 ppm dispersed Mayan crude oil. Length of the fish ranged from 2.9 to 3.9 cm; mean length was 3.4 ± 0.3 cm (Table 1).

After 6 hours of exposure, 85 percent mortality had occurred in 500 ppm dispersed Mayan crude oil. The 6-hour LC_{50} was 418 ppm with 95 percent confidence intervals of 300 to 500 ppm. After 96 hours of exposure, mortality remained at 85 percent in the 500 ppm test concentration, while 30 percent mortality had occurred in the 300 ppm treatment. The 96-hour LC_{50} was 387 ppm dispersed Mayan crude oil, with 95 percent confidence intervals of 300 to 500 ppm. No statistically significant mortality occurred in test concentrations less than or equal to 180 ppm dispersed oil (Tables 9 and 3).

Toxicity tests with dispersed SABL crude oil

Redfish. A flowthrough, 96-hour toxicity test was conducted from February 24 through 28, 1988, using the redfish. Ten fish were tested per duplicate, resulting in 20 fish per test treatment. Test concentrations were 50, 100, 200, 400, and 800 ppm dispersed SABL crude oil. Length of the fish ranged from 2.7 to 4.1 cm; mean length was 3.5 ± 0.4 cm (Table 10). After one hour of exposure, 90 and 95 percent mortality occurred in 400 and 800 ppm dispersed SABL crude oil

Table 10. Test species, test dates, and animal lengths in toxicity tests with SABL crude oil

Test species	Test dates	Animal length (cm) Mean ± SD[1]	Range
Sciaenops ocellatus	24–28 Feb 88	3.5 ± 0.4	2.7–4.1
Penaeus aztecus	29 Apr–03 May 88	3.9 ± 0.9	2.7–5.3
Penaeus aztecus	04–08 May 88	4.2 ± 0.5	3.4–5.0
Penaeus setiferus	09–13 Aug 88	4.1 ± 0.5	3.3–4.9
Penaeus setiferus	09–13 Aug 88	4.1 ± 0.5	3.3–4.9

1. SD = standard deviation

treatments respectively. The 1-hour LC_{50} was 304 ppm, with 95 percent confidence intervals of 200 to 400 ppm dispersed SABL crude oil.

After 6 hours of exposure, 100 percent mortality had occurred in 400 and 800 ppm, and 35 percent in 200 ppm dispersed crude oil treatments. The 6-hour LC_{50} was 226 ppm, with 95 percent confidence limits of 100 to 400 ppm. After 96 hours of exposure, 75 percent mortality occurred in the 200 ppm condition. The 96-hour LC_{50} was 166 ppm dispersed SABL crude oil, with 95 percent confidence limits of 100 to 200 ppm. No statistically significant mortality occurred in test concentrations less than or equal to 100 ppm dispersed oil (Tables 11 and 12).

Brown shrimp. A flowthrough, 96-hour toxicity test was conducted from April 29 through May 3, 1988, using brown shrimp. Ten shrimp were tested per duplicate, resulting in 20 shrimp per test treatment. Test concentrations were 5, 10, 20, 40, and 80 ppm dispersed SABL. Length of the shrimp ranged from 2.7 to 5.3 cm; mean length was 3.0 ± 0.9 cm (Table 10).

After 1 hour of exposure, 70 percent mortality occurred in 80 ppm dispersed SABL crude oil. The 1-hour LC_{50} was 68 ppm dispersed SABL crude oil, with 95 percent confidence limits of 40 to 80 ppm.

After 6 hours of exposure, 100 percent mortality had occurred in 40 and 80 ppm dispersed oil. The 6-hour LC_{50} was 26 ppm, with 95 percent confidence intervals of 20 to 40 ppm. The 96-hour LC_{50} was 23 ppm, with 95 percent confidence intervals of 20 to 40 ppm. No statistically significant mortality occurred in test concentrations less than or equal to 10 ppm dispersed SABL crude oil (Tables 13 and 12).

A static, 96-hour toxicity test was conducted from May 4 through 8, 1988, using the brown shrimp. Ten shrimp were tested per duplicate, resulting in 20 shrimp per test concentration. Test concentrations were 3, 6, 12, 25, and 50 ppm dispersed SABL crude oil. Length of the shrimp ranged from 3.4 to 5.0 cm; mean length was 4.2 ± 0.5 cm (Table 10).

After 1 hour of exposure, 70 percent mortality occurred in 50 ppm

Table 9. Mortality of redfish exposed to dispersed Mayan crude oil in a flowthrough acute test

Oil : dispersant test concentration (ppm)	Rep	Cumulative mortality (percent) 6 h	24 h	48 h	72 h	96 h
Seawater control	A	0	0	0	0	0
	B	0	0	0	0	0
0 : 50	A	0	0	0	10	10
	B	0	0	0	0	0
65 : 6.5	A	0	0	0	0	0
	B	0	0	0	0	0
110 : 11	A	0	0	0	0	0
	B	0	0	0	0	0
180 : 18	A	0	0	0	0	0
	B	0	0	0	0	0
300 : 30	A	0	0	0	0	0
	B	0	30	30	30	30
500 : 50	A	90	90	90	90	90
	B	80	80	80	80	80

Table 11. Mortality of redfish exposed to chemically dispersed SABL crude oil in a flowthrough acute test

Oil : dispersant test concentration (ppm)	Rep	Cumulative mortality (percent)					
		1 h	6 h	24 h	48 h	72 h	96 h
Seawater control	A	0	0	0	0	10	10
	B	0	0	0	0	0	0
0 : 80	A	0	0	0	0	0	0
	B	0	0	0	0	0	0
50 : 5	A	0	0	0	0	0	0
	B	0	0	0	0	0	0
100 : 10	A	0	0	0	0	0	0
	B	0	0	0	0	0	0
200 : 20	A	0	30	50	50	50	50
	B	0	40	100	—	—	—
400 : 40	A	90	100	—	—	—	—
	B	90	100	—	—	—	—
800 : 80	A	90	100	—	—	—	—
	B	100	—	—	—	—	—

Table 12. Summary of results of toxicity tests using chemically dispersed and unaltered SABL crude oil

Test species	Type test[1,2]	LC_{50} value (ppm) or effect measure[3]					
		1 h	6 h	24 h	48 h	72 h	96 h
Sciaenops ocellatus	FT	237	226	166	166	166	166
Penaeus aztecus	FT	68	26	24	23	23	23
Penaeus aztecus	S	43	<3	<3	<3	<3	<3
Penaeus setiferus	S	>16	>16	>16	>16	>16	>16
Penaeus setiferus (oil only)	S	NEM	NEM	NEM	665	224	180

1. S = static
2. FT = flowthrough
3. NEM = not enough mortality to calculate an LC_{50} value

Table 13. Mortality of brown shrimp exposed to chemically dispersed SABL crude oil in a flowthrough acute test

Oil : dispersant test concentration (ppm)	Rep	Cumulative mortality (percent)					
		1 h	6 h	24 h	48 h	72 h	96 h
Seawater control	A	0	0	0	0	0	0
	B	0	0	0	0	0	0
0 : 5	A	0	0	0	0	0	0
	B	0	0	0	0	0	0
5 : 0.5	A	0	0	0	10	10	10
	B	0	0	20	20	20	20
10 : 1	A	0	0	0	0	0	0
	B	0	0	0	0	0	0
20 : 2	A	0	0	40	50	60	60
	B	0	10	10	10	10	10
40 : 4	A	0	100	—	—	—	—
	B	0	100	—	—	—	—
80 : 8	A	70	100	—	—	—	—
	B	70	100	—	—	—	—

dispersed SABL crude oil. The 1-hour LC_{50} was 43 ppm dispersed SABL crude oil, with 95 percent confidence limits of 25 to 50 ppm.

After 6 hours of exposure, 100 percent mortality had occurred in 25 and 50 ppm dispersed oil and greater than 50 percent mortality occurred in all other test concentrations. The 6-hour LC_{50} was less than 3 ppm. After 96 hours of exposure, 100 percent mortality had occurred in all test concentrations. The 96-hour LC_{50} was less than 3 ppm dispersed SABL crude oil (Tables 14 and 12).

White shrimp. A static, 96-hour toxicity test was conducted from August 9 through 13, 1988, using white shrimp. Ten shrimp were tested per duplicate, resulting in 20 shrimp per test concentration. Test concentrations were 1, 2, 4, 8, and 16 ppm dispersed SABL crude oil. Length of the shrimp ranged from 3.3 to 4.9 cm; mean length was 4.1 ± 0.5 cm (Table 10).

After 96 hours of exposure, only 25 percent mortality had occurred in 16 ppm dispersed oil. The 96-hour LC_{50} was greater than 16 ppm. No statistically significant mortality occurred in test concentrations less than or equal to 8 ppm dispersed SABL crude oil (Tables 15 and 12).

Toxicity tests with unaltered SABL crude oil

White shrimp. Conducted concurrently with the dispersed SABL crude oil test of August 9 through 13, 1988, was a white shrimp exposure to unaltered SABL crude oil. Test concentrations were 45, 90, 180, 360, and 720 ppm crude oil. During the first 24 hours of exposure, mortality was insufficient to calculate LC_{50} values. The 48-, 72-, and 96-hour LC_{50}s were 665, 224, and 180 ppm SABL crude oil respectively. After 96 hours of exposure, 100 percent mortality had occurred in the 720 ppm condition. Statistically significant mortality occurred in all test concentrations after 96 hours of exposure (Tables 16 and 12).

Discussion

The objective of the testing program was to determine LC_{50} values for commercially important species indigenous to the Gulf of Mexico. The values were based on a single exposure to the test materials followed by a conservative decrease in test material concentrations. The flowthrough test system worked well in meeting the objective.

The test results indicated that when the test species were exposed to chemically dispersed Mayan or SABL crude oil, the observed tox-

Table 14. Mortality of brown shrimp exposed to chemically dispersed SABL crude oil in a static acute test

Oil : dispersant test concentration (ppm)	Rep	Cumulative mortality (percent)					
		1 h	6 h	24 h	48 h	72 h	96 h
Seawater control	A	0	0	0	0	0	0
	B	0	0	0	0	0	0
0 : 5	A	0	0	0	0	0	0
	B	0	0	0	0	0	0
3 : 0.3	A	0	90	100	—	—	—
	B	0	100	—	—	—	—
6 : 0.6	A	0	60	70	80	100	—
	B	0	40	60	70	100	—
12.5 : 1.25	A	0	100	—	—	—	—
	B	0	90	100	—	—	—
25 : 2.5	A	0	100	—	—	—	—
	B	0	100	—	—	—	—
50 : 5	A	70	100	—	—	—	—
	B	70	100	—	—	—	—

Table 15. Mortality of white shrimp exposed to chemically dispersed SABL crude oil in a static acute test

Oil : dispersant test concentration (ppm)	Rep	Cumulative mortality (percent)					
		1 h	6 h	24 h	48 h	72 h	96 h
Seawater control	A	0	0	10	10	10	10
	B	0	0	0	0	0	0
0 : 1.6	A	0	0	0	0	0	0
	B	0	0	0	0	0	0
1 : 0.1	A	0	0	0	0	10	10
	B	0	0	0	0	0	0
2 : 0.2	A	0	0	0	0	0	0
	B	0	0	0	0	0	0
4 : 0.4	A	0	0	0	0	10	10
	B	0	0	0	0	0	0
8 : 0.8	A	0	0	0	10	10	10
	B	0	0	0	0	0	0
16 : 1.6	A	0	20	20	20	30	30
	B	0	20	20	20	20	20

Table 16. Mortality of white shrimp exposed to unaltered SABL crude oil in a static acute test

Oil test concentration (ppm)	Rep	Cumulative mortality (percent)					
		1 h	6 h	24 h	48 h	72 h	96 h
Seawater control	A	0	0	10	10	10	10
	B	0	0	0	0	0	0
45	A	0	0	10	10	10	20
	B	0	0	0	10	10	20
90	A	0	0	10	10	20	40
	B	0	0	0	20	30	50
180	A	0	0	10	30	40	60
	B	0	0	10	30	20	40
360	A	0	0	0	20	70	90
	B	0	0	20	20	50	100
720	A	0	0	20	60	90	100
	B	0	0	20	50	100	—

icity occurred rapidly, usually within 6 hours after addition of the dispersed oil. It was observed during the tests that the test organisms became disoriented and lethargic immediately after addition of the dispersed crude oils. However, the test animals that survived the first 6 to 12 hours of exposure usually recovered and survived for the remainder of the test period. In almost all cases, the surviving test organisms were swimming normally and were not lethargic 12 to 24 hours after test material addition.

The test results indicated that if the organisms were exposed to dispersed Mayan or SABL crude oil, but survived long enough for the material to decrease in concentration, the organisms could recover and survive without any apparent short-term effects.

References

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2. Stephan, C. E., 1982. Personal communication to Mr. Lowell Bachner, Chairman of the ASTM Task Group on Calculating LC_{50}s (September 10). Communication included computer program and description of program developed by Stephan for calculating LC_{50} values based on his paper "Methods for Calculating an LC_{50}." U.S. Environmental Protection Agency, 6209 Congdon Boulevard, Duluth, Minnesota 55804